

Methods & Techniques

Narcotizing and Fixing Opisthobranchs

by

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Over the years there have been cited a great number of methods for narcotizing and fixing mollusks in general and opisthobranchs in particular. I shall not attempt to review all of these methods but shall state some that I have found to work well on opisthobranchs.

Before discussing the actual methods of narcotizing and fixing opisthobranchs, one must stress the importance of complete field notes, which include careful color descriptions and pattern drawings. The color notes must be made before preservation as there seem to be no methods that will retain the delicate color of opisthobranchs.

When collecting small opisthobranchs from large amounts of algae, a shallow glass tray, filled with fresh sea water and covered with a black cloth, can be used. After one hour the snails will rise to the surface and a few drops of a saturated solution of eucaïne (more stable than cocaine) or menthol crystals can be sprinkled on the surface. In a matter of thirty minutes to one hour the opisthobranchs will be narcotized and can be placed in Gilson's or hot Susa's fixative for two to three hours.

Larger opisthobranchs, such as the Eolids and Elysiidae, should be placed in fresh sea water until they are expanded. Stovaine (amyl chlorohydrin, 1 percent solution) or $MgCl_2$ is gradually added. The relaxed specimens are then placed in Gilson's fixative for about six hours. Dorididae can be narcotized in $MgCl_2$ and killed with boiling Susa's fixative. Aplysiidae may be narcotized with chloral hydrate (a

teaspoon to a medium-sized beaker of water) and placed in liquid of Perényi to harden.

When either Susa's or Gilson's fixatives are used, all traces of the corrosive sublimate must be removed from the opisthobranchs before they are stored in alcohol. This can be accomplished by adding tincture of iodine to 70 percent alcohol containing the specimens. Enough iodine should be added to color the alcohol a port wine shade. The specimens should be placed in changes of fresh alcohol until all traces of the iodine are removed. Do not use metal instruments with either of the above mentioned fixatives; use a paint brush mounted in plastic, or a pipette. For permanent storage 70 percent alcohol should be used. Glycerine can be added in regions of hot weather or when the specimens are to remain packed for some time. This will prevent complete desiccation and decomposition of the specimen in case of evaporation.

Corrosive sublimate (mercuric chloride, bichloride of mercury), as referred to in this article, is a saturated solution in distilled water (about a 7 percent solution). Both Susa's and Gilson's fixatives must be filtered after three days. A list of formulas for the fixatives used in this article is given below.

Susa's Fixative:

$HgCl_2$	45.0 gm.
NaCl	5.0 gm.
Distilled water	800 cc.
Trichloroacetic acid	20 gm.
Acetic acid (glacial)	40 cc.
Formalin (40 percent)	200 cc.

Gilson's mercurio-nitric mixture:

Corrosive sublimate	5.0 gm.
Nitric acid	4 cc.
Glacial acetic acid	1 cc.
Alcohol (70 percent)	25 cc.
Distilled water	200 cc.

Liquid of Perényi:

Nitric acid (10 percent)	4 parts
Alcohol (70 percent)	3 parts
Chromic acid (0.5 percent)	3 parts

